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Award Number: DAMD17-02-1-0358

TITLE: Monitoring Cancer Oxygenation Changes Induced by Ultrasound

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REPORT DATE: July 2003

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2003	Annual Summary (15 Jun 2002 - 14 Jun 2003)

4. TITLE AND SUBTITLE
Monitoring Cancer Oxygenation Changes Induced by Ultrasound

5. FUNDING NUMBERS
DAMD17-02-1-0358

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

This research is aimed at testing a new hypothesis that oxygen delivery to the localized tumor region can be enhanced by ultrasound at the diagnostic radiation level. A new portable frequency-domain multi-channel three-wavelength near infrared (NIR) diffusive imager has been built and calibrated for oxygenated hemoglobin and deoxygenated hemoglobin measurements. A 128-channel ultrasound system has been instrumented for insonification. A total of 3 Fisher rats were injected with the cancer cell line of 9L/Laz (gliosarcoma) and exposed to ultrasound. The ultrasound intensity has been controlled by adjusting the voltage and the pulse repetition frequency. Dramatic changes were observed consistently in one rat, and further experiments are planned to verify the findings.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Cancer therapy, cancer hypoxia, optical spectroscopy, ultrasound contrast agent	8
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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INTRODUCTION:

Breast cancer ranks second among cancer deaths of women in United States, and over 210,000 new cases of breast cancer are expected to occur during 2003 alone [1]. The treatment of breast cancer includes surgery, radiation, chemotherapy, and hormonal modification [2]. In the treatment for solid malignancies by radiation therapy, the most important modulator of response is the presence of oxygen. The presence of oxygen can prolong the lifetime of ionized molecules and thereby increase the radiosensitivity of a cell by up to a factor of 3 [3]. Chemotherapeutic efficacy may also be adversely affected by oxygen deprivation. Recent studies show that hypoxia may have a profound impact on malignant progression and responsiveness to therapy [4]. Attempts to improve hypoxic cell oxygenation may therefore have positive effect on outcome of cancer treatment by radiation, chemotherapy, or the combination thereof.

The purpose of this research is to test a new hypothesis that oxygen delivery to the localized tumor region can be increased by the microbubbles exposed to ultrasound at the diagnostic radiation level. Microbubbles are safely used as ultrasound contrast agents to enhance the tumor vessel visualization [5-7]. When the microbubbles are exposed to ultrasound at diagnostic radiation level, they experience primary radiation force which will deflect a large percent of them toward the vessel wall [8-10]. The microbubble deflection may change the permeability of tumor vessels. The increase in tumor vascular permeability and volume will enhance the oxygen diffusion to the localized tumor region, which in turn will increase the efficacy of cancer therapy.

In this research, a new portable frequency-domain multi-channel multi-wavelength near infrared (NIR) diffusive imager has been built and calibrated for oxyHb and deoxyHb measurement for the monitoring of cancer oxygenation changes. A 128-channel ultrasound pulser has been instrumented for insonification. Prior to the proposed study on the hypothesized permeability change of tumor vessels by microbubble deflection, animal experiments with rat tumor models have been setup to assess the NIR system performance and possible oxygen diffusion changes induced by ultrasound alone [11-13].

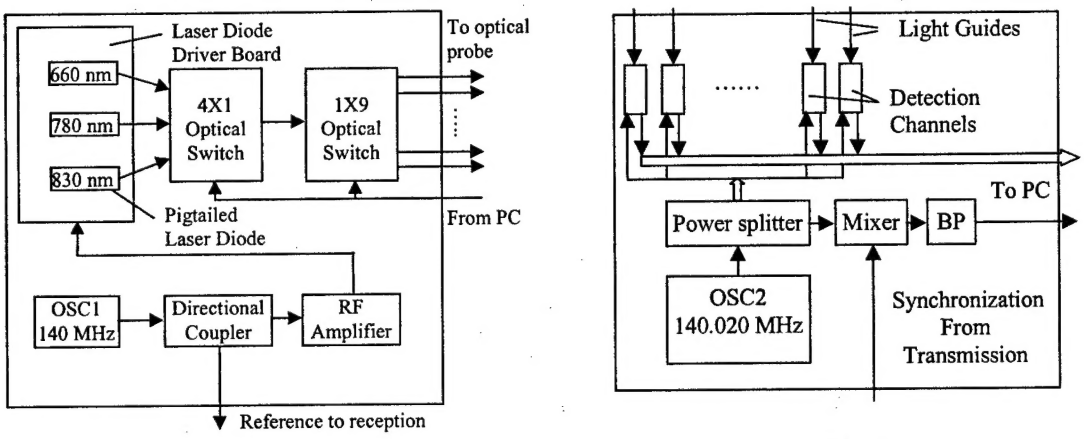
In the next step, we will modify our experiment by putting an additional NIR detection channel to monitor the oxygenation changes in normal tissue as a reference to remove common-mode fluctuations. We will continue the animal experiments with rat tumor models under insonification only and with microbubbles. We will investigate the experimental data; and look into further problems emerging from animal experiments.

BODY:

Multi-channel three-wavelength NIR imager

From the initial studies with five rats, we have observed that the detected light signals at different tumor locations vary a lot, particularly for larger tumors. We do have an existing NIR imager with multiple detection channels for monitoring the changes, however, the system is used at the University of Connecticut Health Center for clinical studies. In addition, the existing system is very heavy and bulky, which makes the transportation difficult. We have to develop a new NIR diffusive optical tomography system for animal studies. The existing NIR imager was equipped with 12 dual wavelength (780 nm and 830

nm) laser diode source pairs and 8 PMT detectors. The newly developed NIR imager features three-wavelength excitations, fast optical switching in 9 transmission channels, and 8 high-dynamic range avalanche photodiode (APD) detectors. In the transmission part (see Fig. 1(a)), pigtailed laser diodes at 660, 780, and 830 nm are used as light sources and are amplitude modulated at 140 MHz. Compared with the existing NIR imager, wavelength excitation at 660nm is added to better extract average absorption background of H_2O. One 4X1 and one 1X9 optical switches are combined to form a 4X9 switch, which distributes the output of one wavelength to one of nine source fibers. The short switching time (about 3 ms) keeps the data acquisition within 1 second for a complete scan. This makes it possible to monitor dynamic physiological changes such as oxygen diffusion or perform near real time tomographic imaging in the later stage of this research. The crosstalk between channels is around 60 dB, equivalent to 120 dB in opto-electrical signals. In the reception part (see Fig. 1(b)), eight Silicon APD's detect diffusive photon density waves simultaneously. Since the active area of each APD has a diameter of 1.5 mm, an aspherical lens is used to couple the diffusive light collected by a 3 mm diameter light guide to an APD. The dynamic range of APD's is several orders higher than that of PMT's, which is essential for imager using reflection geometry. Since the internal gain of the APD's we are using is 3 orders less than ordinary PMT's, efforts have been made to suppress the feed through interferences from the transmission part to the receiving part so as to reduce the errors in amplitude and phase measurements.



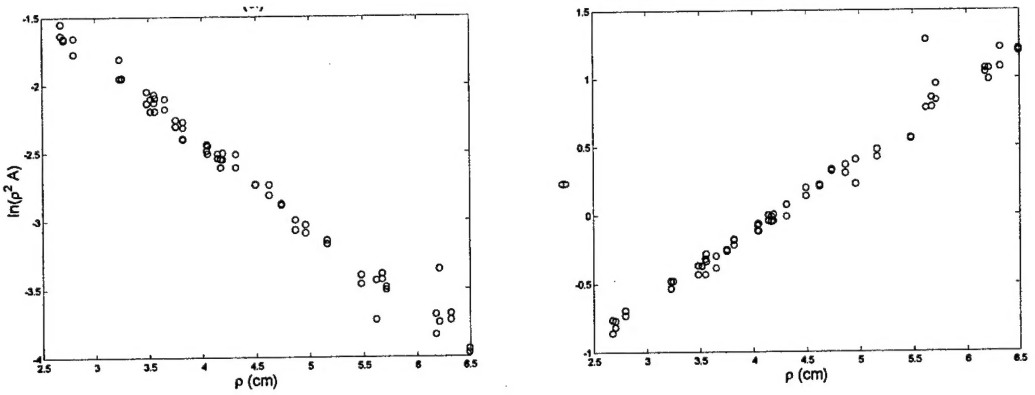
(a) (b)

Figure 1. Schematic of new NIR imager. (a) Transmission part, (b) reception part.

Our new imager has been calibrated using 0.52% Intralipid solution that has optical properties similar to human breasts. By use of a calibration method described in our previous publication [14], relative gains and phase delays can be obtained as well as corrected amplitudes and phases. Shown in Fig. 2 are functions of amplitude and phase versus distance after calibration. The reconstructed optical properties of the Intralipid solution are listed in Table 1. We also tested the performance of this imager with spherical targets positioned inside the Intralipid solution. The reconstructed images indicate the right location, distribution, and values of both absorption and diffusion heterogeneities.

Table 1. Absorption and reduced scattering coefficients at multiple wavelengths.

Wavelength	660 nm	780 nm	830 nm
Absorption coefficient (cm^{-1})	0.0048	0.0212	0.0245
Reduced scattering coefficient (cm^{-1})	5.54	5.35	5.36



(a) (b)

Figure 2. Amplitude (a) and phase (b) vs. source-detector distance after calibration

128-Channel ultrasound pulser

In this study we used a donated planar ultrasound transducer of 196 usable elements functioning at 7.5 MHz. Only 128 of the channels were used because the ultrasound pulser is instrumented with 128-channel. The pulser can provide 200 Volts peak-to-peak pulses and up to 2K repetition rate. A program was developed based on LabVIEW programming where the sub arrays within the ultrasonic transducer elements can be chosen, steered and focused to produce the desired beam in the needed locations.

Preliminary animal experiments

A total of 3 Fisher rats **were injected** with the cancer cell line of 9L/Laz (gliosarcoma). The rats had been monitored for about 2 weeks before the tumors grow to a decent size to be experimented on. A bendable ring-probe was made to house and stabilize up to 3 NIR sources and 4 detectors around the tumor regions. The tumor region enclosed by the NIR probe was covered with ultrasound coupling gel, and immerged in the gel is the ultrasound transducer tip placed above the tumor region. A picture of the setup is shown in Fig. 3. After the NIR imager is stabilized, the ultrasound excitation was initiated and the transmission of the acoustical wave was administered on the tumor region while the NIR system picked up any changes in oxygenation with/without insonfication.

The rats were administered with different ultrasonic intensity levels by adjusting the voltage and the pulse repetition frequency to see how the intensities would affect the oxy/deoygenation measurements. Clear changes were observed consistently in one rat subject, however, at this point, it is hard to draw conclusions. What can be verified from the data is that when the ultrasound pulse of 180v p-p and 2kHz pulse-repetition-rate (prf) was applied, NIR signal changes may occur during earlier cancer stages, but not in the later cancer stages. One such change is shown in Fig. 4. In order to better extract the oxygenation changes by ultrasound excitation in the tumor region, we plan to modify our experimental setup to use one more NIR channel as a reference to detect the normal

tissue instead of tumor region so that any common fluctuation in the measurement can be suppressed.

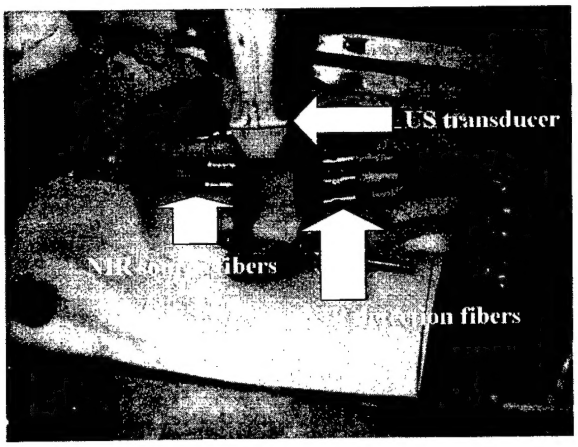


Figure 3. The animal experimental setup. Picture shown here is taken before applying the US gel

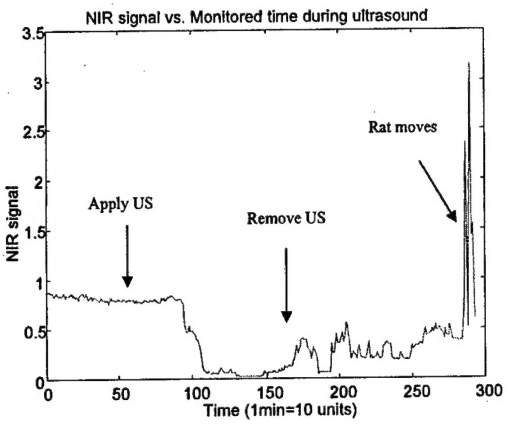


Figure 4. NIR signal monitoring before and after insonification.

KEY RESEARCH ACCOMPLISHMENTS:

- Built a new diffusive NIR imager with multiple channels and three wavelengths;
- Calibrated and optimized the new optical imagers for oxyHb and deoxyHb measurements;
- Assisted in the study of imaging algorithms;
- Assisted in the instrumentation and testing of the ultrasound system;
- Studied ultrasound system parameters toward maximum oxygenation changes;
- Did preliminary animal experiments to investigate ultrasound-induced oxygenation changes
- Investigated the analysis of the preliminary data.

REPORTBALE OUTCOMES:

Presentations:

[1] N. G. Chen, D. Piao, H. Xia, and Q. Zhu, "Portable multi-channel multi-wavelength near infrared diffusive light imager," SPIE Photonics West 2003, San Jose, January, 2003.

[2] M Huang, T. Xie, N. G. Chen, D. Piao, and Q. Zhu, " 2-D NIR image reconstruction with ultrasound guidance," Proceedings, 2002 IEEE International Symposium on Biomedical Imaging, pp1031-1034 (Washington D.C., 2002).

CONCLUSIONS:

I have successfully finished the Task 1 and partial of Task 2 and Task 3 specified in the statement of work of my proposal. I have built, tested, and calibrated a new portable multi-channel three-wavelength NIR diffusive imaging system. I have also assisted in the study of NIR imaging algorithms and the instrumentation of a 128-channel ultrasound pulser. I have conducted animal experiments to investigate perhaps ultrasound-induced

oxygenation changes prior to the proposed microbubble experiments. The significant instrumentation effort, parameter optimization study and preliminary animal experiment experience will substantiate my future investigations.

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